

Supplemental Data

Article

Discovery of Drug-Resistant and Drug-Sensitizing Mutations

in the Oncogenic PI3K Isoform p110 α

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Supplemental Experimental Procedures

YRP1 Transformation

YRP1 was transformed with plasmid DNA by electroporation with a protocol adapted from D. Gottschling, Fred Hutchinson Cancer Research Center, Seattle: <http://www.fhcrc.org/science/labs/gottschling/yeast/ytrans.html>. Briefly, YRP1 were grown in 50 ml YPD medium at 30°C until early stationary phase ($OD_{660} = 1.05-1.25$) and then harvested by centrifugation at 3000 RPM, 4°C, for 5 minutes. The cells were then washed twice with ice-cold sterile distilled H₂O, centrifuging at 2500 RPM, 4°C, for 5 minutes, and washed once with ice-cold 1 M Sorbitol, centrifuging at 2000 RPM, 4°C, for 5 minutes. The cells were then resuspended with 1.5 ml ice-cold 1 M Sorbitol, and 80 μ l of the cell suspension was mixed with 2 μ l plasmid DNA prep (0.5-2 μ g) and transferred to a pre-chilled 0.1 cm gap electroporation cuvette (BioRad Genepulser), and electroporated with the following settings: 1.5 kV Voltage, 25 μ F Capacitance, 200 Ω Resistance. Immediately after electroporation, the cells were suspended in 1 ml ice-cold 1 M Sorbitol and transferred to a pre-chilled 1.5 ml eppendorf tube, before spreading onto selective media. Transformation efficiencies of 1×10^5 colonies/ μ g DNA were routinely observed, greatly improving on the 1×10^2 colonies/ μ g DNA efficiency observed with standard lithium acetate transformation.

Supplemental References

Hayakawa, M., Kaizawa, H., Kawaguchi, K., Ishikawa, N., Koizumi, T., Ohishi, T., Yamano, M., Okada, M., Ohta, M., Tsukamoto, S., *et al.* (2007). Synthesis and biological evaluation of imidazo[1,2-a]pyridine derivatives as novel PI3 kinase p110 α inhibitors. *Bioorg Med Chem* 15, 403-412.

Knight, Z. A., Gonzalez, B., Feldman, M. E., Zunder, E. R., Goldenberg, D. D., Williams, O., Loewith, R., Stokoe, D., Balla, A., Toth, B., *et al.* (2006). A pharmacological map of the PI3-K family defines a role for p110 α in insulin signaling. *Cell* 125, 733-747.

Stauffer, F., Maira, S. M., Furet, P., and Garcia-Echeverria, C. (2008). Imidazo[4,5-c]quinolines as inhibitors of the PI3K/PKB-pathway. *Bioorg Med Chem Lett* 18, 1027-1030.

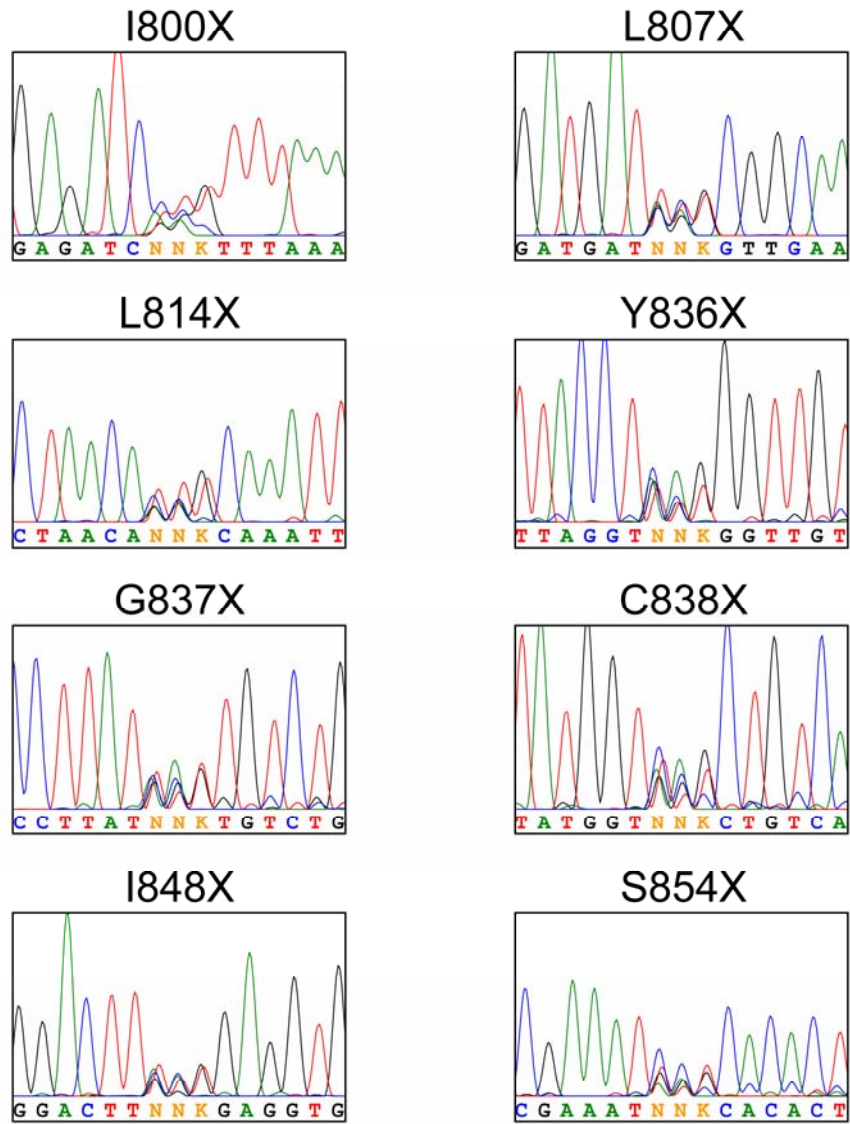


Figure S1. DNA Sequencing Chromatograms of the p110 α Mutagenic Libraries

Randomized codons of the p110 α affinity pocket NNK libraries, sequenced with the following primer: (5'-3') ACCCAGATCCTATGGTTCGAGG.

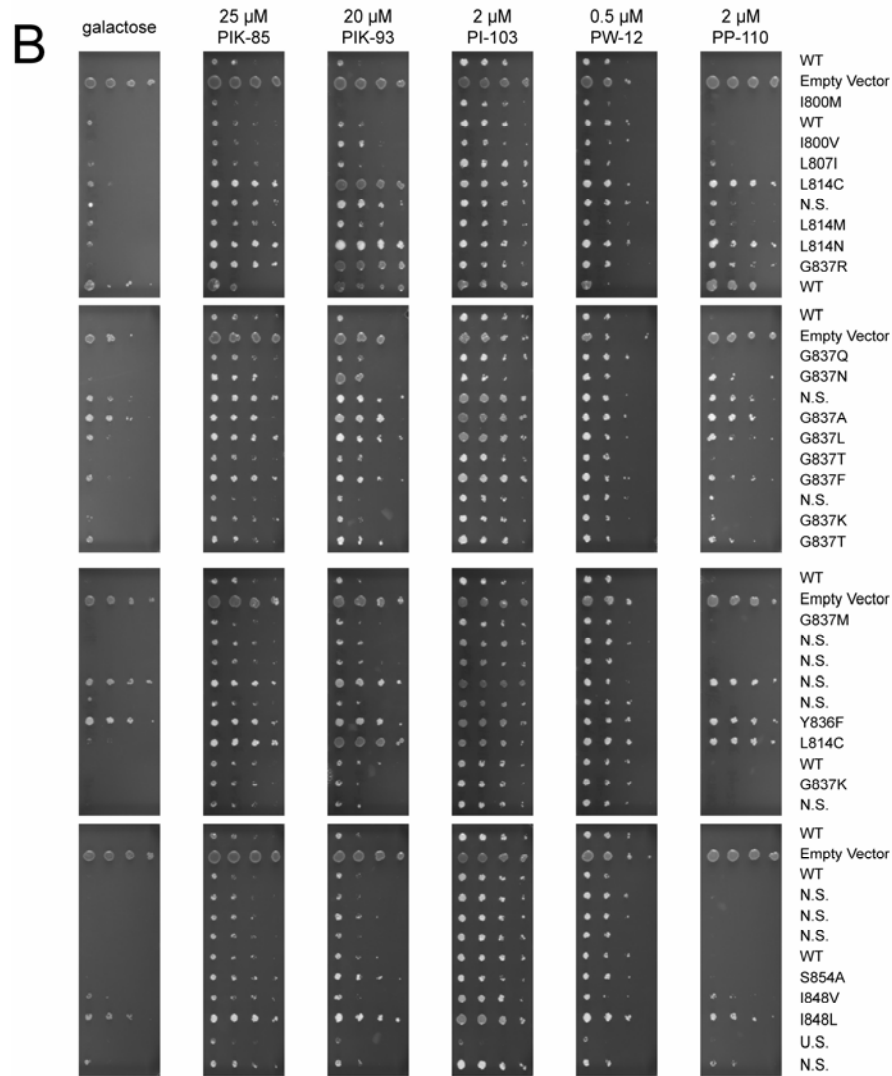
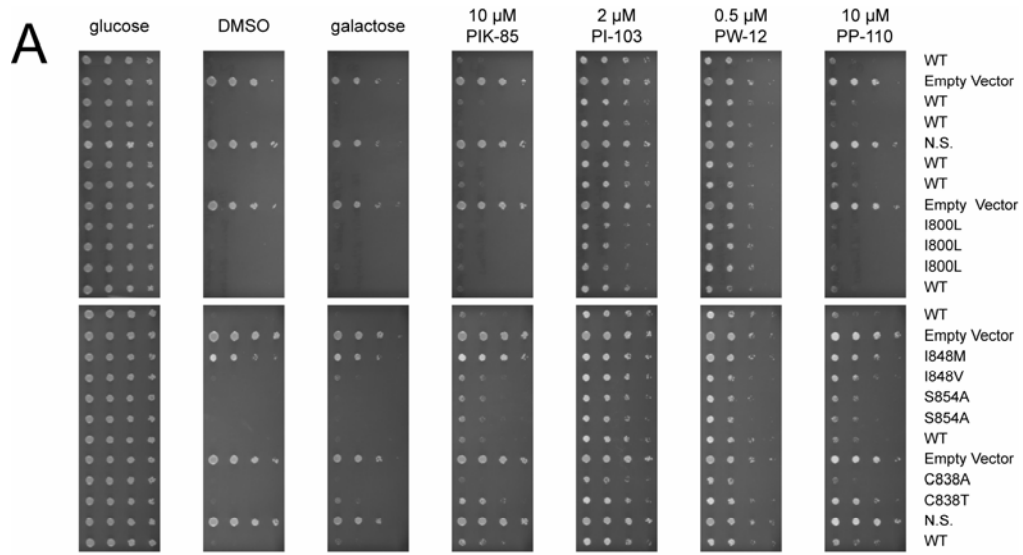


Figure S2.

Figure S2. Characterization of Yeast Screen Hits by Serial Dilution Analysis

Six-fold serial dilutions of YRP1-p*URA3*-2 μ -*GAL1*-p110 α H1047R-CAAX strains with the indicated p110 α mutations spotted onto agar plates of SD –uracil medium containing either glucose or galactose. The plates were incubated at 30°C for 2 days (glucose) and 6 days (galactose). Strains that were not sequenced are labeled N.S. (not sequenced). Strains that were unable to be sequenced due to plasmid rescue failure are labeled U.S. (unable to sequence).

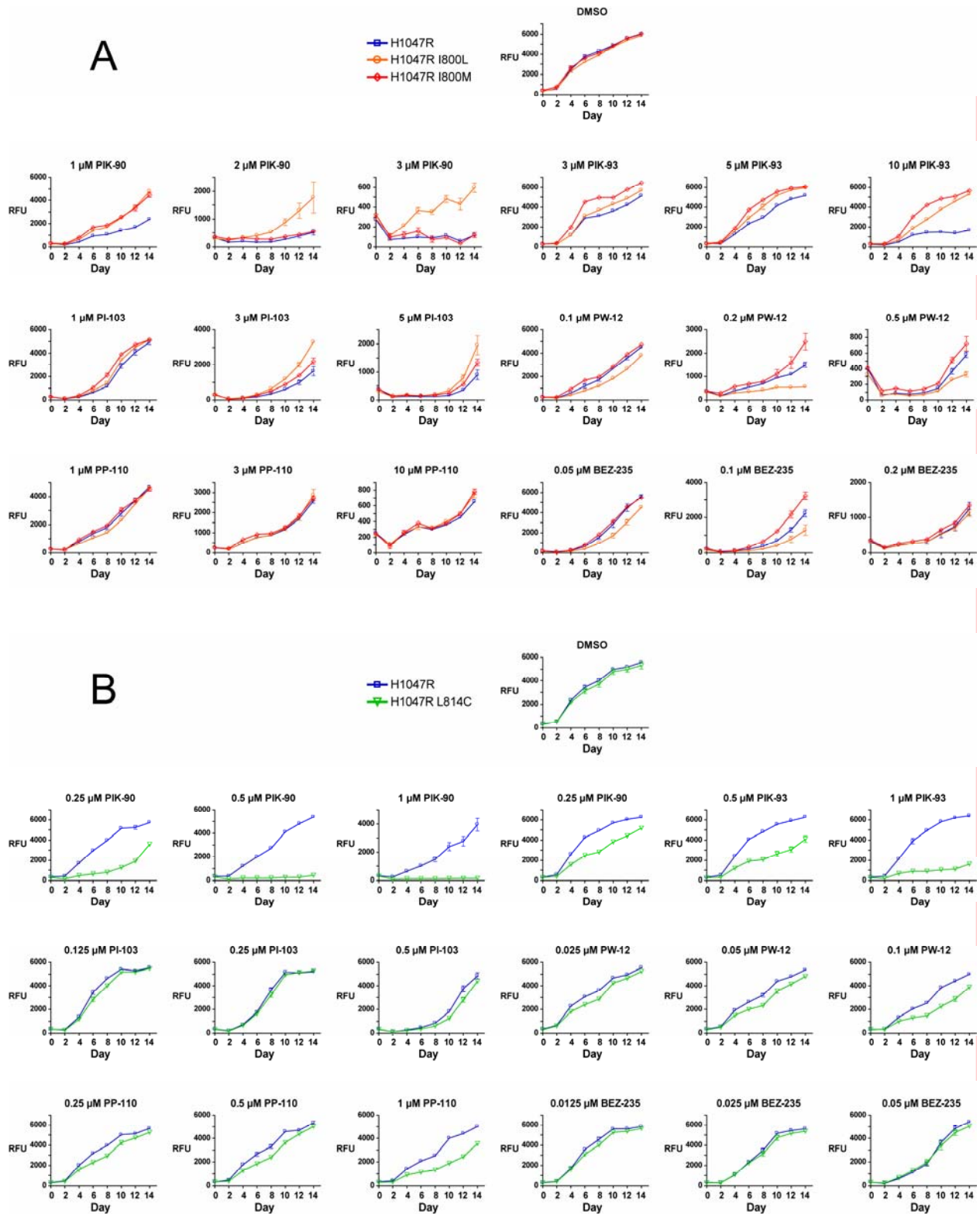


Figure S3.

Figure S3. p110 α H1047R-Transformed MCF-10A Growth Curves at Additional Drug Concentrations

Growth of the I800L, I800M, and L814C MCF-10A cell lines in medium lacking EGF was monitored as in Figure 6A in the presence of the indicated PI3K inhibitors. Data are represented as mean \pm SEM.

Table S1. Inhibitor Concentrations Used in the p110 α Mutant Screen

	Low	Medium	High
PIK-85	2 μ M	10 μ M	20 μ M
PIK-93	2 μ M	10 μ M	20 μ M
PI-103	1 μ M	2 μ M	5 μ M
PW-12	100 nM	500 nM	2 μ M
PP-110	2 μ M	10 μ M	not done

Table S2. Inhibitory Values for PI3K Inhibitors against p110 α and Off-Target Protein Kinases

	p110 α	Off-Target Protein Kinases
PIK-90	0.011 μ M IC ₅₀	
PIK-93	0.039 μ M IC ₅₀	
PI-103	0.008 μ M IC ₅₀	0.02 μ M IC ₅₀ @ 100 μ M ATP (mTORC1) 0.083 μ M IC ₅₀ @ 100 μ M ATP (mTORC2)
PW-12	0.003 μ M IC ₅₀ @ 1 μ M ATP	91% inhibition at 1 μ M (CDK1/cyclin B) 94% inhibition at 1 μ M (FLT3) 89% inhibition at 1 μ M (Fyn) 92% inhibition at 1 μ M (GSK3 β) 91% inhibition at 1 μ M (PKC α)
PP-110	0.069 μ M IC ₅₀	0.03 μ M IC ₅₀ (Hck) 0.069 μ M IC ₅₀ (Src)
BEZ-235	0.004 μ M IC ₅₀	low nM IC ₅₀ (mTOR)

This table was assembled with published inhibitory values (Hayakawa et al., 2007; Knight et al., 2006; Stauffer et al., 2008) (B.A. and K.M.S., unpublished data) and experimentally determined results. All inhibitory values were determined at 10 μ M ATP unless otherwise indicated.

Table S3. Oligonucleotide Primers Used for Cloning and Site-Directed Mutagenesis

	Primer Sequence (5'-3')
hp110 α -FM	cctctatactttaacgtcaaggagaaatgctccacgaccatcatcaggtgaactg
hp110 α -CRM	gcgcgccatcaagagagcacacacttacagttcaatgcatgctgttaattgtgtgg
hp110 α -FL	gtatcaacaaaaattgtaatactctatactttaacgtcaagg
hp110 α -CRL	cgcgtaatacgaactactatagggcgaattgggtacccccggcgcctcatcaagagagc
hp110 α R1047H-F	aatgatgcacATcatggtggctggacaacaaaaatgg
hp110 α R1047H-R	ccagccaccatgATgtgcatcattcattgtttc
hp110 α K802R-F	gagatcatctttCGaaatggggatgattacggc
hp110 α K802R-R	ccccatttCGaaagatgatctcattgttctgaacagtaactc
p110 α -BamHI-NtermMyc-F	cttctggtaccatggaacagaaactatcatcgaggaggatctacctccacgaccatcatcaggtgaactgtgg
p110 α -EcoRV-R	ccgcatatctcagttcaatgcatgctgttaattgtgtgg
hp110 α F-XhoI	gtgctttgctcgagatgctccacgaccatcatcaggtgaactg
hp110 α R-HpaI	gtgcctcggttaactcagttcaatgcatgctgttaattgtgtgg

Table S4. Oligonucleotide Primers Used for Site-Directed NNK Mutagenesis

	Primer Sequence (5'-3')
I800-Forward	caatgagatcNNKtttaaaaatgggatgatttacggcaagatatgc
I800-Reverse	catttttaaMNNgatctcattgttctgaacagtaactctgacatgatgtctgg
L807-Forward	gggatgatNNKcggcaagatatgtaaacacttcaaattattcg
L807Reverse	catatcttgccgMNNatcatccccatttttaagatgatctcattg
L814-Forward	gatagctaacaNNKcaaattattcgtattatgaaaaatatctggc
L814-Reverse	cgaataattgMNNtggtagcatacttggcgtaaatcatcccc
Y836-Forward	cgaatgttacctNNKgggtgtctgcaatcgggtgactgtgtgggac
Y836-Reverse	ccgattgacagacaaccMNNaggtaacattcgaagatcaagacc
G837-Forward	cgaatgttacctatNNKtgtctgcaatcgggtgactgtgtgggac
G837-Reverse	ccgattgacagacaMNNataaggtaacattcgaagatcaagacc
C838-Forward	cgaatgttacctatggtNNKctgtcaatcgggtgactgtgtgggac
C838-Reverse	ccgattgacagMNNaccataaggtaacattcgaagatcaagacc
I848-Forward	tgtgggacttNNKgaggtgtgcaaaattctcactattatgc
I848-Reverse	gcaccacctcMNNaagtcacacagtcaccgattgacagac
S854-Forward	gtgcgaaatNNKcactattatgcaaaattcagtgcaagggcggc
S854-Reverse	gcataatagtgtMNNatttcgaccacctcaataagtc